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Trefoil factor-2, an early predictor for acute gastrointestinal injury in patients with acute pancreatitis

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Abstract

Acute gastrointestinal injury (AGI) is commonly present in patients with acute pancreatitis (AP). It is often difficult to predict gastrointestinal function in the early stage due to lack of reliable markers. We aimed to assess whether early plasma trefoil factor 2 (TFF-2) is a potential predictor for AGI.

Fifty one patients were included for the onset of AP (from developing abdominal pain) within 72 hours in this prospective observational single-center study from January 2013 to July 2015. Among them 23 patients were classified as mild, 17 as moderately severe, and 11 as severe according to 2012 Atlanta classification. Plasma samples were collected only once at admission to the ICU. Twenty samples of healthy adults were also collected as control. The TFF-2 levels were determined by using a human TFF-2 enzyme-linked immunoassay. AGI grades from 1st to 7th day after admission were observed.

The plasma TFF-2 levels among AP patients in early stage were significantly higher than healthy controls (766.41 ng/mL vs 94.37 ng/mL, $P < .0001$). The correlations between TFF-2 levels and AGI grades from 1st to 4th day after admission were positive ($r = 0.47$, 0.43, 0.42, 0.40 respectively, $P < .05$). As a predictor of acute gastrointestinal failure, plasma TFF-2 was superior to others: Acute Physiology and Chronic Health Evaluation II, sequential organ failure assessment, procalcitonin, C-reactive protein, serum calcium. In addition, TFF-2 increased along with the severity of AP ($r = 0.554$, $P < .0001$) and associated with Acute Physiology and Chronic Health Evaluation II, sequential organ failure assessment, C-reactive protein, serum calcium.

The plasma TFF-2 levels were increased in patients in early stage of AP and correlated with AGI grades and disease severity in our study. TFF-2 might be a potential predictor for acute gastrointestinal failure in patients with AP.

Abbreviations: ACS = abdominal compartment syndrome, AGI = acute gastrointestinal injury, AP = acute pancreatitis, APACHE-II = Acute Physiology and Chronic Health Evaluation II, BMI = body mass index, CRP = C-reactive protein, ELISA = Enzyme-linked immunoassay, FI = feeding intolerance syndrome, ICU = intensive care unit, MODS = multiple organ dysfunction syndrome, PCT = procalcitonin, ROC = receiver operating characteristic, SD = standard deviation, SIRS = systemic inflammatory response syndrome, SOFA = sequential organ failure assessment, TFF-2 = trefoil factor 2.

Keywords: acute gastrointestinal failure, acute gastrointestinal injure, acute pancreatitis, trefoil factor 2

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R-LX and W-WC contributed equally to this article.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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1. Introduction

Acute gastrointestinal injury (AGI) is common in patients with acute pancreatitis (AP). Approximately 59% of AP patients have experienced gastrointestinal (GI) dysfunction during their hospital stay.[1] The GI tract has various functions including digestion and absorption, endocrine, immune, and barrier function.[2] GI dysfunction significantly contributes to the translocation of microbes and their products, which can lead to the initiation of systemic inflammatory response syndrome (SIRS), development of sepsis, multiple organ dysfunction syndrome (MODS), and even death.[3] Thus, early accurate identification of patients at risk for GI injury would allow appropriate clinical management to improve prognosis. The evaluation of GI function mainly consists of the markers of GI barrier or GI mucosal integrity, such as intestinal fatty acid binding protein, Citrulline, Diamine Oxidase, Lactulose/Manntiol Ratio, and bacterial translocation (Endotoxins, Endo-CAb),[1] which could not reveal the degree of GI injury during the acute phase due to instable diagnostic accuracy.

The trefoil factor family (TFFs) is comprised of 3 peptides (TFF-1, TFF-2, TFF-3), which are characterized by 3-looped structural motifs held together by disulphide bounds.[4] TFFs are highly expressed in systemic tissue containing mucous epithelia, especially in the GI tract.[5] TFF-2 was the first TFF molecule identified. It contains 106 amino acids (12KD) residing in 2 homologous trefoils domains which help resistance against acid and upper GI proteases.[6] TFF-2 is mostly expressed in the upper GI glands, but also in pancreas and biliary ducts, and lower expression in small intestine and colon.[5,7] TFF-2 is rapidly secreted by whole GI epithelia after GI injury and through multiple mechanisms to protect and restitute GI damaged mucosa.[5] These mechanisms might be as follows[5]: anti-apoptosis, promote epithelial cells migrate to cover impaired surfaces, interact with its unique mucin to enhance the mucosal barrier,[8] regulated by both pro-inflammatory and anti-inflammatory cytokines expression and participate in inherent immune reaction.[9]

The previous studies of TFF-2 were predominantly focused on GI malignancies and chronic inflammatory diseases. Recently, Shah et al[9] has observed that bacterial dissemination to distant organs in TFF-2 deficient mice infected by *Yersinia enterocolitica*. Žurek et al[10] has found that the levels of TFF-2 were significantly higher in septic children with MODS. It seems that TFF-2 may be related to bacterial translocation and development of sepsis and MODS result from GI defense dysfunction. However, the potential role of TFF-2 in acute GI damage caused by AP has not been determined. We therefore studied whether early plasma TFF-2 is a potential predictor for GI dysfunction and whether plasma level of TFF-2 associates to the severity of AP.

2. Methods

2.1. Patients

All patients (age 18–75 years) were included only for the onset of abdominal pain <72 hours and first admission to IUC of our hospital. Diagnosis of AP was based on typical clinical symptoms (acute onset of a persistent, severe, epigastric pain often radiating to the back) with serum amylase activity at least 3 times greater than the upper limit of reference and/or characteristic findings of AP on computed tomography.[11] Patients were excluded if any of the followings were present: a history of chronic pancreatitis; confirmed or suspected malignancy; immune deficiency or immunodepression; end-stage chronic diseases; chronic inflammatory disease: asthma, peptic ulcer, inflammatory bowel disease; pregnancy or lactation.

This study enrolled 51 patients with AP admitted to the Emergency ICU and Surgery ICU from January 2013 to July 2015. As controls, the blood samples (n = 20) were collected from healthy adults who did physical examination in Ruijin hospital. The hospital medical ethics committee approved this clinical study (Reference No: Clinical Trial No.49, 2013). Each patient or their next of kin and healthy participants were informed and gave consent.

2.2. Definitions

The severity of acute pancreatitis was classified dynamically as mild, moderately severe, and severe, according to the 2012 Atlanta classification.[11] The severity grades of AGI and the definitions of feeding intolerance syndrome (FI), GI symptoms (vomiting, diarrhea, paralysis, high gastric residuals, abnormal bowel sounds, bowel dilatation), intra-abdominal hypertension, and abdominal compartment syndrome (ACS) according to the 2012 Recommendations of the European Society of Intensive Care Medicine Working Group on Abdominal Problems.[2] The AGI grading was assessed once a day by 3 senior doctors respectively until the patient was discharged or died.

The grades of AGI of AGI were identified[2]: Grade 0 is absence of AGI; Grade I stands for a self-limiting condition with increased risk of developing GI dysfunction or failure; Grade II (GI dysfunction) is a condition requiring interventions to restore GI function; Grade III (GI failure) is a condition when GI function cannot be restored with interventions; Grade IV is dramatically manifesting GI failure, which is immediately life-threatening. We defined AGI Grade I and Grade II as the absence of acute gastrointestinal failure (AGF), while Grade III and Grade IV as the existence of AGF.

Acute Physiology and Chronic Health Evaluation II (APACHE-II) were used to measure the severity of AP during the first 24 hours after admission. Sequential organ failure assessment (SOFA) on admission was used to assess organ failure. The levels of C-reactive protein (CRP), procalcitonin (PCT), and serum calcium were used to predict the severity of AP.

2.3. Blood samples

Venous blood samples were collected only once at admission to the intensive care unit (ICU) when AP diagnosis was established. Samples were centrifuged at 3000 rpm for 10 minutes and separated plasma was stored at −80 °C until biochemical analysis.

2.4. Biochemical analysis

TFF-2 levels were quantitative tested by a human TFF-2 (hTFF-2) enzyme-linked immunoassay (ELISA) (Westang Bio-Tech Co. Shanghai, China). TFF-2, CRP, PCT, and serum calcium were performed on the same plasma sample. CRP, PCT, serum calcium were determined by the hospital laboratory. The laboratory technicians performing the assays were completely blinded to the clinical information.

2.5. Statistical analysis

Normally distributed continuous data are presented as mean ± standard deviation (SD). Non-normally distributed data are

Table 1

Comparison between AP patients and healthy controls.

Variable	AP patients (n = 51)	Healthy controls (n = 20)	*P* value
Age, y (median, IQR)	42 (29–51)	44 (32–55)	.925*
Female, n (%)	20 (39.22)	8 (40.0)	.943†
BMI, kg/m^2	25.41 ± 4.54	22.23 ± 7.41	.075*
TFF-2, ng/mL Median (IQR)	766.41 (425.75–1638.42)	94.37 (43.51–313.63)	<.0001*

AP = acute pancreatitis, BMI = body mass index, IQR = inter quartile range, TFF-2 = trefoil factor 2.
* Wilcoxon rank test.
† Fisher exact test.

presented as median (25th percentile to 75th percentile). Categorical data are presented as n (%). Comparisons between 2 groups were tested with Wilcoxon rank test and between 3 or more groups with Kruskal-Wallis test. The Fisher exact test was used for all comparisons of proportions. Correlations between 2 variables were using Spearman rank correlation. Diagnostic accuracy was portrayed as the area under the receiver operating characteristic (ROC) curve (AUC) and try to find out optimal cut-off values of TFF-2, sensitivity, and specificity were given. Level of significance was considered $P < .05$. All statistics were conducted in SAS (version 9.2, SAS Inc., Cary, NC).

3. Results

Plasma samples for TFF-2 analysis were collected on median 48 (36–64) hours after the onset of symptom. TFF-2 levels among patients were significantly higher than healthy controls (766.41 ng/mL vs 94.37 ng/mL, $P < .0001$). There is no significant difference between patients and controls of age, sex, and body mass index (Table 1).

3.1. The association between TFF-2 levels and grades of AGI

AGI grades were assessed daily in the first 7 days after admission. The correlations between the plasma TFF-2 levels and AGI grades from 1st to 4th day after admission were positive. The coefficient of correlation is 0.47, 0.43, 0.42, and 0.40 respectively; *P* value is .001, .001, .002, and .004 correspondingly (Fig. 1).

3.2. The predictive value of TFF-2 and other predictors for acute gastrointestinal failure

The plasma levels of TFF-2, the serum levels of PCT, CRP, calcium, and SOFA scores on admission, APACHE-II scores in first 24 hours after admission, were plotted in ROC curve to measure their predictive value of GI dysfunction (Fig. 2). The diagnostic accuracy of TFF-2 (0.809, 95% CI 0.634–0.985) is higher than other predictors. The AUC of APACHE-II, SOFA, CRP, PCT, and serum calcium was 0.777 (95% CI 0.602–0.951), 0.723 (95% CI 0.534–0.913), 0.694 (95% CI 0.513–0.874), 0.522 (95% CI 0.331–0.712), and 0.680 (95% CI 0.459–0.902), respectively. The optimal cut-off values and sensitivity and specificity of each predictors based on ROC curves are shown in Table 2.

3.3. Assessment of severity and AGI grades of AP patients on admission

Fifty one AP patients with 23 (45.10%) were classified as mild, 17 (33.33%) as moderately severe, and 11 (21.57%) as severe according to 2012 Atlanta classification. There is no significant difference of age, sex, body mass index, and etiology among 3 groups of mild acute pancreatitis (MAP), moderately severe acute pancreatitis (MSAP), and severe acute pancreatitis (SAP) (Table 3).

TFF-2 levels were the highest in patients who developed SAP (1809.86 ng/mL; inter quartile range [IQR] 770.91–4448.87), which were higher than MSAP patients (1437.04 ng/mL; IQR 589.71–2868.25), and the lowest in patients who were MAP (471.25 ng/mL; IQR 302.63–682.29) (Table 3). The positive relation between plasma TFF-2 levels and severity of AP was identified ($r = 0.55$, $P < .0001$; Fig. 3A). In addition, the plasma levels of TFF-2 were positively correlated with APACHE-II

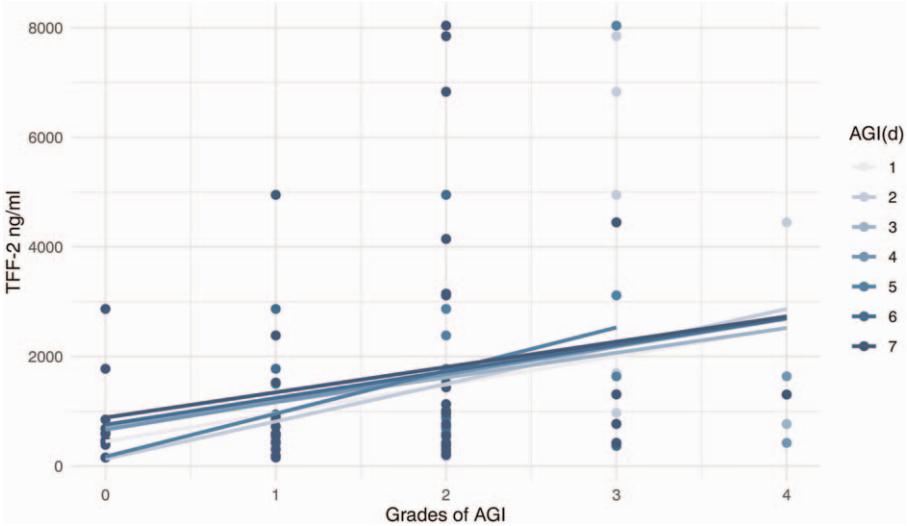

Figure 1. The levels of TFF-2 in venous blood samples were immediately tested on admission when AP diagnosis was established, while AGI score were assessed in 7 consecutive days. The positive correlation of TFF-2 levels at admission with AGI grades from 1st to 4th day. The coefficient of correlation is 0.47, 0.43, 0.42, and 0.40 respectively; *P* value is .001, .001, .002, and .004 correspondingly. AGI = acute gastrointestinal injury, AP = acute pancreatitis, TFF-2 = trefoil factor 2.

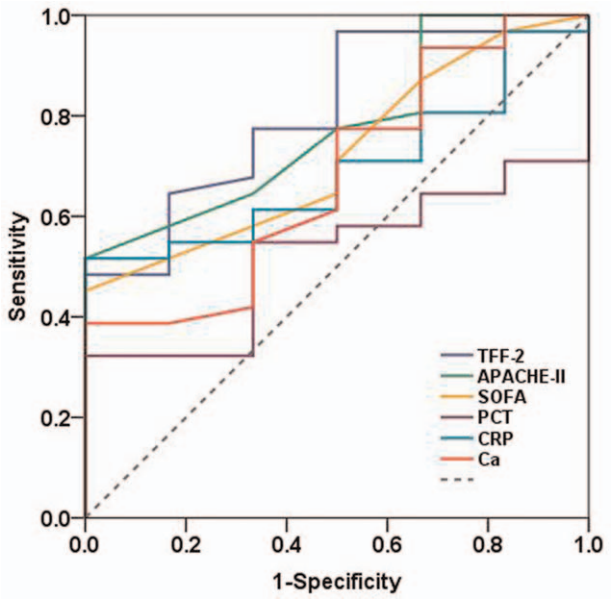

Figure 2. Value of trefoil factor 2 (TFF-2), Acute Physiology and Chronic Health Evaluation II (APACHE-II) at first 24 hours after admission, sequential organ failure assessment (SOFA) on admission, C-reactive protein (CRP), procalcitonin (PCT), and serum calcium (Ca) in predicting acute gastrointestinal failure in the first week of AP. The area under the receiver-operating characteristic curve (AUC) of TFF-2, APACHE-II, SOFA, CRP, PCT, Ca was 0.809, 0.777, 0.723, 0.694, 0.522, 0.680, respectively.

Table 2

Predicting value of TFF-2 and common clinical indicators for acute gastrointestinal failure in early stage of AP.

Predictors	Cut-off	Sensitivity	Specificity	AUC (95% CI)
TFF-2, ng/mL	≥591.83	0.645	0.833	0.809 (0.634–0.985)
APACHE-II	≥7	0.518	0.833	0.777 (0.602–0.951)
SOFA	>4	0.452	1.000	0.723 (0.534–0.913)
CRP, mg/L	≥104.00	0.548	0.833	0.694 (0.513–0.874)
PCT, μg/L	≥1.215	0.323	1.000	0.522 (0.331–0.712)
Ca, mmol/L	≤1.725	0.387	1.000	0.680 (0.459–0.902)

APACHE-II = Acute Physiology and Chronic Health Evaluation II, AUC = area under the receiver-operating characteristic curve, Ca = serum calcium, CI = confidence interval, CRP = C-reactive protein, PCT = procalcitonin, SOFA = sequential organ failure assessment, TFF-2 = trefoil factor 2.

during the 24 hours after admission ($r = 0.41$, $P = .003$; Fig. 3B), SOFA on admission ($r = 0.34$, $P = .015$; Fig. 3C), CRP ($r = 0.31$, $P = .004$; Fig. 3D). The inversed correlation was found between TFF-2 and serum calcium ($r = -0.35$, $P = .012$; Fig. 3E). There is no correlation between TFF-2 and PCT ($r = 0.18$, $P = .28$; Fig. 3F).

4. Discussion

The major finding of our study is that early plasma TFF-2 levels were not only associated with the severity of AGI, but also associated with the severity of AP. It suggests that plasma TFF-2 may be able to predict accurately GI dysfunction.

We found that early plasma levels of TFF-2 among patients were significantly higher than healthy persons (766.41 ng/mL vs 94.37 ng/mL). This finding may be explained by that during the

Table 3

Assessment of severity and AGI grades of AP patients on admission.

Variable	MAP (n = 23)	MSAP (n = 17)	SAP (n = 11)	*P* value
Age, y Median (IQR)	42 (31–47)	42 (34–54)	38 (25–51)	.7366[a]
Female, n (%)	10 (43.48)	7 (41.18)	3 (27.27)	.7043[b]
BMI, kg/m^2	24.83 ± 4.96	26.62 ± 4.07	24.77 ± 4.33	.4159[a]
Etiology, n (%)				.6147[b]
Biliary	13 (56.52)	5 (29.41)	5 (45.45)	–
Hypertriglyceridemia	7 (30.43)	8 (47.06)	5 (45.45)	–
Alcohol	2 (8.70)	2 (11.76)	0 (0)	–
Other*	1 (4.35)	2 (11.76)	1 (9.09)	–
Severity of AP				
TFF-2, ng/mL Median (IQR)	471.25 (302.63,682.29)	1437.04 (589.71,2868.25)	1809.86 (770.91,4448.87)	.0003[c]
APACHE-II in 1st 24 h	4.87 ± 2.88	9.47 ± 3.76	19.82 ± 5.04	<.0001[a]
SOFA on admission	2.70 ± 1.52	3.82 ± 1.91	8.36 ± 2.62	<.0001[a]
CRP, mg/L	77.65 (34.85,115.5)	152 (117,192)	192 (106,237)	.0186[c]
PCT, μg/L	0.29 (0.05,0.66)	0.51 (0.24,1.03)	6.69 (2.34,22.75)	0.0061[c]
Ca, mmol/L	2.0 ± 0.15	1.66 ± 0.36	1.30 ± 0.35	<.0001[a]
Severity of AGI n (%)				<.0001[b]
Grade 0	2 (8.70)	0 (0)	0 (0)	
Grade I	8 (34.78)	0 (0)	0 (0)	
Grade II	13 (56.52)	15 (88.24)	2 (18.18)	
Grade III	0 (0)	2 (11.76)	5 (45.45)	
Grade IV	0 (0)	0 (0)	4 (36.36)	

AGI = acute gastrointestinal injury, AP = acute pancreatitis, APACHE-II = Acute Physiology and Chronic Health Evaluation II, Ca = serum calcium, CRP = C-reactive protein, IQR = inter quartile range, MAP = mild acute pancreatitis, MSAP = moderately severe acute pancreatitis, PCT = procalcitonin, SAP = severe acute pancreatitis, SOFA = sequential organ failure assessment, TFF-2 = trefoil factor 2.

* Other: Medication, Idiopathic, etc.

[a] Analysis of variance.

[b] Fisher exact test.

[c] Kruskal-Wallis test.

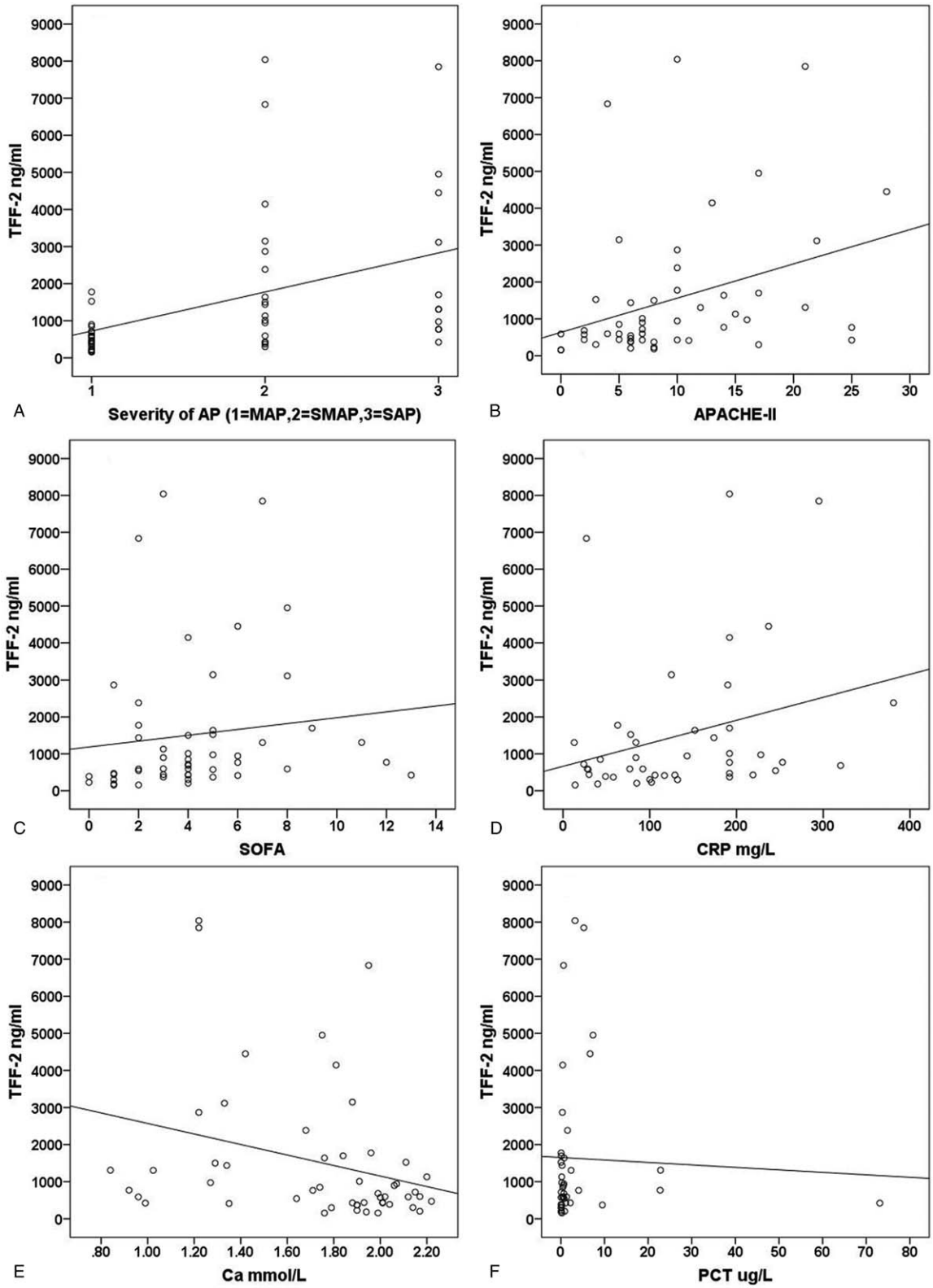

Figure 3. (A) Positive correlation of plasma levels of TFF-2 with severity of acute pancreatitis (AP) ($r = 0.55$, $P < .0001$). (B) Positive correlation of plasma levels of TFF-2 with Acute Physiology and Chronic Health Evaluation II (APACHE II) ($r = 0.41$, $P = .003$). (C) Positive correlation of plasma levels of TFF-2 with sequential organ failure assessment (SOFA) ($r = 0.34$, $P = .015$). (D) Positive correlation of plasma levels of TFF-2 with C-reactive protein (CRP) ($r = 0.31$, $P = .004$). (E) Inversed correlation of plasma levels of TFF-2 with serum calcium (Ca) ($r = -0.35$, $P = .012$). (F) No correlation of plasma levels of TFF-2 with procalcitonin (PCT) ($r = 0.18$, $P = .28$). TFF-2 = trefoil factor 2.

early phase, AP is not merely local pancreatic inflammation, but also is an initiator of systemic inflammatory response.[11] It reduces splanchnic perfusion, resulting in GI ischaemia and epithelial barrier integrity loss.[1,12] Previous research has shown that intestinal permeability is increased as early as 48 to 72 hours of the onset of AP.[13] TFF-2 is a small peptide (12 kD), which is rapidly expressed and secreted in the whole GI tract after gut injury.[5] Also, it is easily released into circulation through the damaged gut barrier leading to circulating concentration elevated.[5,14] To some extent the presence of TFF-2 in GI tissue is mirrored by their presence in plasma.[14] Thus, we consider that the AP patients with higher plasma TFF-2 levels might suffer GI injury.

In 2012, the Working Group on Abdominal Problems of the European Society of Intensive Care Medicine proposed the guidelines for the grading system of AGI.[2] Until now, clinical studies[15,16] reported that AGI grading system is feasible and effective in identify severity of GI damage in critically ill patients, but it still lacks the support of quantitative indicators. Our results show that TFF-2 levels have moderately positive correlation with AGI grades after admission from 1st day to 4th day; probably the reason for no strong correlation is that the blood samples were few, but to some degree it still shows that the TFF-2 levels were associated with AGI. After admission, patients were treated with fluid resuscitation, gastrointestinal decompression, inhibition of gastric acid and pancreatin, enteral nutrition, organ support if any and so on. These treatments relieved GI injury and the grades of AGI were improved correspondingly. This phenomenon might explain the correlation between early plasma TFF-2 and AGI Grades was eliminated after 4 days of admission. Proton-pump inhibitor or somatostain might have an impact on TFF-2 synthesis and secretion,[5,17] but it was not shown in our study because of the blood samples were collected at admission and before treatment. Each patient have received standard treatments according to guideline,[11] thus there is no effect on individualized difference to AGI assessments.

As mentioned above, early plasma TFF-2 levels were associated with the severity of AGI during the early phase of AP onset. TFF-2 may be an objective marker to provide a preliminary assessment of GI dysfunction. This result was also supported by the higher AUC value (0.809) than other scores (APACHE-II 0.777, SOFA 0.723) and parameters (CRP 0.694, PCT 0.522, Ca 0.680). We choose 591.83 ng/mL (Sensitivity 0.645, Specificity 0.833) as optimal cut-off value of TFF-2, through maximizing the sum of sensitivity and specificity, to determine AP patient with GI dysfunction. This value can be the reference value for our further study.

We also found that as Atlanta classification of AP was more serious, the level of plasma TFF-2 was higher. Moreover, the positive correlation between plasma TFF-2 and some commonly used clinical measurements of disease severity, such as APACHE-II, SOFA, CRP, were identified.[18–20] Serum calcium, which has shown similar efficacy like APACHE-II in prediction of SAP,[18] was correlated inversely with TFF-2. Therefore, the plasma levels of TFF-2 could help predicting the severity of AP in the early phase. It is consistent with previous findings that the degree of GI permeability correlated with the severity of pancreatitis[12] and GI failure is associated with impaired outcomes.[21,22]

We didn't find any association between TFF-2 and PCT. During the early phase of AP, which usually lasts for the first week, cytokine cascades are activated by the pancreatic inflammation and lead to system immune disorder.[11] The GI tract is the vulnerable organ, but also the "motor" of the subsequent development of sepsis and MODS.[3] Bacterial infection and local complications evolve during the late phase.[11] The role of PCT as a biomarker for sepsis and bacterial infection is well documented.[23] It up-regulated at 2 to 4 hours post-bacterial infection and has a half-life of 22 to 26 hours.[24] Thus, it is reasonable that TFF-2 and PCT has no correlation since they were secreted in different phase.

To our knowledge, this is the first clinical report of plasma TFF-2 for predicting AGI in patients with AP. However, there are still a few of limitations of our study. The small sample size may influence on our results, but if the sample enlarged, the diagnostic accuracy of plasma TFF-2 levels could be even greater. Plasma collections were only performed on admission, rather than continuous dynamic monitoring of TFF-2 levels. Therefore, the predictive value of TFF-2 will be expected in a further study.

In conclusion, the plasma TFF-2 levels were increased in patients with AP in early stage and correlated positively with AGI grading and disease severity. TFF-2 might be a potential predictor for AGF in AP patients during the early phase.

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